THE METABOLISM OF ZINC IN THE SEX GLANDS AND ADRENALS OF THE MALE RAT USING ZN^{65} AS A TRACER

by

Allan Dennis Rudzik, B. Sc.

EDMONTON, ALBERTA

August, 1958

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ABSTRACT

A study was made of the effects of adrenalectomy and hypophysectomy on zinc concentration and zinc^{65} incorporation in a number of tissues of the male rat. The tissues studied were the whole blood, liver, adrenals, testis, dorsolateral and ventral prostate.

The effects of the administration of adrenocorticotrophic hormone and cortisone were also observed.

Adrenalectomy caused significant decreases in the zinc concentration of blood and dorsolateral prostate with a return to normal following chronic treatment with cortisone.

Cortisone caused increased levels of Zn^{65} in adrenalectomized animals in all tissues except the testis, following chronic administration of the hormone.

Hypophysectomy caused the zinc concentration to be decreased in dorsolateral prostate with a corresponding decrease in Zn^{65} incorporation.

Chronic treatment of hypophysectomized animals with ACTH resulted in a lowering of zinc concentration in all tissues but increased the Zn^{65} incorporation in dorsolateral and ventral prostate.

In normal animals treated with cortisone daily the adrenal glands showed a marked increase in the zinc concentration and Zn^{65} incorporation. In normal animals treated with ACTH daily there was a decrease in the zinc concentration but an increased relative specific activity. Hypophysectomy resulted in an initial decrease (at 5 days) in zinc concentration but an increase after 19 days with a significant decrease in the relative specific activity. It is postulated that zinc is in some way associated with corticosteroid formation.

Since adrenalectomy produced a decrease in the zinc concentration of the dorsolateral prostate which was essentially the same as that produced by hypophysectomy and treatment with cortisone in chronic dosage caused a return to normal, (with a concomitant increase in relative specific activity), it is postulated that the adrenal gland is associated with zinc metabolism of this tissue.

THESIS
1958(F)
#24

THE UNIVERSITY OF ALBERTA

THE METABOLISM OF ZINC IN THE SEX GLANDS AND ADRENALS OF THE MALE RAT USING ZN^{65} AS A TRACER

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCE

FACULTY OF PHARMACY

by

Allan Dennis Rudzik, B.Sc.

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ACKNOWLEDGEMENTS

The author gratefully acknowledges the valuable assistance, the encouragement and constructive criticism offered by Dr. B.E. Riedel throughout the course of this investigation.

He wishes to thank the National Research Council for financial assistance given to Dr. B.E. Riedel in support of this research.

He is grateful to the Merck-Sharpe Dohme Co. Ltd. for supplying the Cortisone Acetate used in these experiments.

His thanks are also extended to Miss L. Frylich for technical assistance, Miss J.E. Zelmer for statistical analysis, Mr. J. Gula for care of experimental animals, and Mrs. F.S. Glenfield for typing the manuscript.

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1. INTRODUCTION

Studies on the metabolic importance of trace elements have become of increasing interest. Zinc is classed as a trace element and has been found to be present in many tissues, in different species of animals, and in different concentrations in different tissues (1).

The significance of the presence of zinc has not yet been completely established. It is apparent that certain enzymes (2-6) require zinc, but in some tissues such as the dorsolateral prostate the amount of zinc present cannot be accounted for solely by the enzymes.

Progress in the field of trace element studies has been slow because of limited physical and chemical techniques which could be applied to the estimation of minute quantities and because of the dynamic nature of the elements being studied.

The more recent application of radio-isotopes in this field has permitted much more extensive studies of the changes occurring in these elements.

Studies on the distribution of zinc in animal tissues by Mawson and Fischer (7) in 1951 showed that the dorsolateral prostate gland contained a greater amount of zinc than any other organ in the rat, rabbit, and man. Mawson, Fischer, and Riedel in 1955 demonstrated the concentration of zinc (Zn^{65}) in the dorsolateral prostate gland of the rat after the intravenous administration of the isotope (8). This was also demonstrated by Gunn, Gould, Ginori and Morse

(9) in 1955. They found that the dorsolateral prostate of the rat concentrated Zn^{65} from 15 to 25 times more than any other organ.

Studies with radioactive zinc by Gunn and Gould (10) have shown the relative importance of androgen and estrogen in the selective uptake of Zn^{65} by the dorsolateral prostate of the rat.

Millar, Elcoate and Mawson (11) found that gonadotrophins and testosterone propionate, administered by subcutaneous injection into immature rats, produced marked increases in the size, zinc concentration, and rate of zinc uptake by the dorsolateral prostate gland.

Gunn and Gould (12) also found that the efficiency of the dorsolateral prostate of the rat to concentrate Zn^{65} was markedly depressed following hypophysectomy but this glandular function can be maintained in the absence of the pituitary by administration of suitable doses of chorionic gonadotrophin or testosterone.

In the work presented here a study has been made of the relationship of the adrenal gland and the pituitary to the distribution of radioactive zinc in certain tissues of the rat. Normal animals have been compared with adrenalectomized and hypophysectomized animals. The effects of adrenocorticotrophic hormone and cortisone have been observed in these groups of animals.

It is postulated that the adrenal gland is associated with the control of zinc metabolism in the tissues studied. It has previously been shown that the gonadotrophins have a marked effect on zinc metabolism in the dorsolateral prostate. The work reported here, however, has shown that in the presence of the pituitary gland, changes in adrenal activity also result in certain changes in the dorsolateral zinc picture.

2. LITERATURE SURVEY

A. Zinc in Nutrition.

Zinc is a constituent of most foods and is necessary for normal growth and development of both plants and animals (13). Rats fed on a diet low in zinc soon exhibit a characteristic deficiency syndrome (14). Growth is retarded, nutrition is impaired, alopecia develops and death may occur within four weeks (15-22). Hyperkeratinization of the skin and extreme parakeratosis of the oesophagus are the only outstanding pathological changes (23-24).

The enzymatic activity of the tissue of zinc deficient rats is significantly reduced. The enzymes affected are intestinal and blood phosphatases, carbonic anhydrase, and hepatic and renal catalases (15-18,25).

Ingestion of large amounts of zinc on the other hand leads to microcytic and hypochromic anaemia, hyperglycemia, subnormal growth, reproductive failure and liver atrophy in young rats (26-31). Fibrotic changes occur in the pancreas after large doses (32).

Wachtel, Hove, Elvehjem, and Hart (33) found that the uric acid content of zinc deficient rat's blood was doubled on all diets tested. Carbohydrate, fat, protein, or nucleoprotein content of the diet do not influence the course of zinc deficiency. Sadasivan (25,30) observed an increase in urinary and faecal nitrogen excretion as well as an increased faecal excretion of phosphorus and sulfur in

animals on a diet supplemented with zinc. The urinary excretion of phosphorus and sulfur decreased, however. Urinary excretion of uric acid and creatinine also increased. No change in the faecal excretion of fat was observed.

Zinc is indispensible in mouse and rat physiology, and several human pathological states are accompanied by or may be related in some way to zinc deficiency (35).

B. Excretion and Absorption.

In 1927, Drinker, Fehnel and Marsh (36) reported on the zinc content of normal human urine and faeces. They indicated that one mg. of zinc was excreted in 24 hours in the urine and about ten mg. in the faeces over a similar period. There was a rise in faecal excretion when zinc rich foods were consumed. McCance and Widdowson (37) state that the normal intake is 10 to 15 mg. per day. Only about 0.3 mg. per day was excreted in the urine. The amount of the urinary output appears to be independent of intake. In albuminuria the urinary zinc rose to 2.1 mg. per day. This loss was not compensated for by diminished faecal excretion.

In 1954, Tribble and Scoular (38) studying zinc metabolism in young college women receiving a diet containing 9.8 to 14.4 mg. of zinc daily found that 8 per cent was excreted in the urine and 42 per cent in the faeces per day.

Using Zn^{65} as an indicator Sheline et al (39) found that in mice 50 per cent of the injected dose appeared in the faeces in 170 hours while only 2 per cent appeared in the urine. In dogs 25 per cent of the dose appeared in the faeces after 12 to 14 days and 1.2 to 4.7 per cent in the urine.

Large portions of the injected Zn^{65} was eliminated by external secretion of the pancreas (40-41). At the same time very little appeared in the bile or duodenal juices. Feaster, Hansard, McCall and Davis (52) found the absorption from the gastrointestinal tract poor (only 7.9 per cent). There was free movement across placenta to the fetus at all stages of gestation.

C. Tissue Zinc Amounts.

Investigators have attempted to determine the quantities of zinc in various invertebrates including man as a possible clue to its function(7, 43-70). It is difficult however, to compare the results of earlier workers to those of recent times because of the diversity of methods and the variation in units used to express concentration.

Choosing those data which refer to wet weight, it is apparent that zinc is present in all human and other vertebrate organs in quantities from 10 to 200 micrograms per gram. Most organs including the pancreas contain from 20 to 30 micrograms per gram. Liver, muscle, and bone contain about double this amount and lymph nodes and endocrine glands contain from 60 to 180 micrograms (35). Until 1951, however, none of the investigators considered the genital organs. Mawson and Fischer (7) found that the dorsolateral prostate of rat contained more zinc than any other tissue except bone. This observation has been substantiated by the findings of Gunn et al (9) who found that the dorsolateral prostate concentrates more Zn^{65} than any other organ.

Gunn and Gould (71) have also differentiated between the dorsal and lateral components of the dorsolateral prostate. It is interesting to note that the ventral prostate contains very little zinc (7).

Many workers have determined the zinc content of human and other vertebrate blood, but the results when compared are confusing because of the different methods used and the different units chosen, (46, 54,55,62,64-69, 72).

There is no variation in zinc levels with age or sex. Three quarters of the blood zinc was found in erythrocytes, 22 per cent in plasma and 3 per cent in leukocytes.

It has been found by Keilin and Mann (2) that all the zinc in the red blood cells is associated with the carbonic anhydrase molecule.

Though only 3 per cent of the total blood zinc is contained in leukocytes, cell for cell they contain twenty-five times as much zinc as do erythrocytes. The absence of uniform distribution in the various components of blood suggests that zinc has a physiological role in blood.

The discovery of zinc in relatively large amounts in leukocytes is surprising but the role of zinc in leukocytes is completely unknown and there are no clues as to its possible function (55,64,65). Attempts at radioactive tagging in order to study leukocytic life span have been inconclusive (73,74).

The nature of the bond between plasma proteins and zinc is not understood completely at present (54,62,69). Wolff (69) has determined that 20 to 30 per cent of zinc in the blood is combined with albumin, and 20 to 30 per cent with α and β globulin.

Mann (76) has investigated the iron, zinc and copper content of ram semen. Zinc occurred in a higher concentration than the other two elements. Mawson and Fischer (57) stated that the highest concentration is found in the first fraction of ejaculate which is mainly composed of prostatic secretion.

D. Zinc in Pathological Conditions.

1. Cancer

Interest in the relation of zinc to cancer developed as a result of the reports concerning the occurrence of zinc in many human and vertebrate organs and that certain tumours were particularly rich in zinc (46, 76). Because of the unreliable techniques used this work is of value only insofar as it may have stimulated interest in the field.

The experimental production of teratomata in the cock's testis by injection of zinc chloride or zinc sulfate has been reported (77-80). Periods of high normal or induced sexual activity seemed necessary for the effectiveness of these injections (78).

This work prompted Bischoff to investigate the possible carcinogenic role of zinc (81). He found that zinc injections into the breast of a mouse bearing inbred adenocarcimomata of the breast, actually inhibited the development of carcinoma as compared with injections of normal saline into controls. Sugai (82, 83) reported that malignant tissues have a very high zinc and very low copper content. Recent work by Mawson and Fischer (58) do not confirm these findings.

Addink (84) found that patients with malignant tumours had a higher serum zinc content then normal. Vikbladh (67, 68)

however, has found that patients with leukemia have a significantly decreased zinc content in serum. The same was true in the case of patients with malignant tumours.

Vallee and Altschule (35) have reported that the zinc content of leukemic leukocytes was depressed to a tenth of the normal value. Under successful therapy with x-ray and urethane the zinc content of leukocytes rose to normal levels as the leukocyte count fell. Injection of stable zinc compounds failed to influence the progress of the disease or raise the zinc level of the leukemic cells.

Several workers have studied the zinc content in epidermal carcinoma and have reported the zinc content to be greatly reduced from normal (85,87).

Using Zn^{65} as an indicator investigators have found a higher concentration in tumour tissue than in normal tissue (45,88,89). It is, however, unlikely that Zn^{65} could be used in tumour therapy since other tissues involved would receive too high a dosage of Zn^{65}.

2. Other Pathological Conditions

Scott and Fisher (50,60,90,91) evoked much controversy by their report on the zinc and insulin content of normal and diabetic pancreas. They reported values of 0.14 mg. of zinc per gram of tissue for normal pancreas and 0.07 mg. of zinc per gram of tissue for diabetic pancreas.

Eisenbrand and Sienz (92) found values of only 30.6 micrograms per gram of fat-free tissue for normal and 25.3 micrograms per gram fat-free tissue for diabetic pancreas. These values are compatible with those of other investigators.

Vikbladh (68) could not find any disturbance in zinc serum content in patients with diabetes mellitus.

In pernicious anemia the zinc content of erythrocytes was found to be significantly elevated before therapy with liver was instituted (66,93,94). After the institution of therapy there was a continuous fall of the zinc content to normal levels after 60 to 70 days. Vallee (93) reported that the zinc content in anemias due to iron and dietary deficiency were within normal limits.

Vikbladh (68) found normal serum zinc levels in all 8 cases of essential iron deficiency anemia studied, but in pernicious anemia before liver therapy the serum zinc levels were markedly lowered. Twenty four hours after the first injection of liver the zinc content began to rise, usually reaching a maximal value during the first week.

Vikbladh (68) studied the serum zinc content in various diseases. He found that cases of acute rheumatic fever showed normal serum zinc levels while cases of chronic polyarthritis showed a significative decrease. Patients with ulcerative colitis showed low serum zinc content. In seventeen cases with hepatogenic icterus a significant decrease in the serum zinc content was noted. Two cases of hyperthyroidism exhibited elevated serum zinc levels. Subjects with chronic nephritis had low serum zinc values and those with acute nephritis had normal values.

Other worker (95,96) have observed lowered serum zinc levels in myocardial infarction, and in patients with

cirrhosis of the liver.

Wolff (69) made an extensive study of serum zinc levels in various pathological conditions and found elevated levels in hyperthyroidism, essential hypertension, polycythemia vera, eosinophilia, and x-radiation. Serum zinc levels were decreased in anemia, leukemia, tumourous infections, liver disease and cardiac insufficiency. His values agree closely with the previous observations of Vikbladh (68).

E. Zinc Complexes.

The crystallization of insulin as a zinc salt (97) led to the belief that zinc is an integral part of the insulin molecule. Hallas - Møller and Peterson (98) found that the zinc concentration is an important factor in the action of insulin. Solutions of insulin with 0.1 per cent zinc had a prolonged effect on the blood sugar level (99).

Various workers have observed an augmentation in the effect of gonadotrophic extracts when zinc salts were added (100-103). These effects were not observed when the hormones themselves were injected.

It seems certain that zinc uroporphyrin exists. Kapp (104) found that a combined form of coproporphyrin which occurs in the human urine is a zinc compound.

Six zinc metalloproteins have been characterized thus far: the carbonic anhydrase of ox erythrocytes (2), the zinc containing protein of human leukocytes (105-107), the carboxypeptidase of bovine pancreas (3), the alcohol dehydrogenase of yeast (4), rabbit muscle lactic dehydrogenase (5), and glutamic dehydrogenase of beef liver (6).

1. Carbonic Anhydrase.

Carbonic anhydrase is the enzyme which catalyzes

the following reaction:

$$H_2CO_3 \rightleftarrows H_2O + CO_2$$

Keilin and Mann (2,108) found conclusive proof that zinc was the prosthetic group of the carbonic anhydrase molecule. They found that a parallelism existed between carbonic anhydrase activity and zinc content. The removal of zinc from the molecule resulted in an inactivation of the enzyme. There appeared to be no other metal associated with the molecule. Keilin and Mann found the zinc content to be 0.3 to 0.33 per cent. Scott and Fisher (68) only observed 0.2 to 0.23 per cent zinc in the carbonic anhydrase molecule.

Tupper et al (109) kept carbonic anhydrase in a solution of $zinc^{65}$ containing zinc salts and found that it takes up very little zinc. When erythrocytes were labelled in vitro with Zn^{65} little or none of the zinc which entered the erythrocytes was firmly attached to carbonic anhydrase. Therefore it would appear that the metal is an integral part of the molecule and is firmly bound to protein.

Carbonic anhydrase is also found in the parietal cells of gastric mucosa of cats and rats (110), in the prostate gland of rats, rabbits and humans (111), and also in plants (112).

There is no significant decrease in carbonic anhydrase in zinc deficient rats (113).

2. Leucocyte Zinc Protein.

Since no carbonic anhydrase could be detected in leukocytes (105), the work of purification of the zinc-containing protein of leukocytes (54,106,107,114) was undertaken to

determine the physiological and biochemical significance of zinc in human leukocytes. Human leukocytes were found to contain zinc in a constant proportion to the total content of protein. The zinc present was firmly bound to protein in a ratio of 82-117 µg of zinc per gram of protein. Under the conditions of extraction used by Hoch and Vallee (54) a soluble fraction comprising 81.1 per cent of zinc of leukocytes and 79.6 per cent of protein could be obtained. The remaining 20.4 per cent of leucocyte protein was insoluble under the conditions of extraction. Zinc was not associated with fractions of nucleic acids.

3. Pancreatic Carboxypeptidase.

Vallee and Neurath (3) have demonstrated that carboxypeptidase is a zinc metalloenzyme containing 1 atom of zinc per molecule of enzyme protein. The zinc content increases as the activity increases, becomes constant with crystallization, and is not materially altered by recrystallization. Other metals present decrease to very minute amounts on recrystallization. The zinc-protein bond is maintained through the changes in pH, ionic strength, temperature and against competition with other ions. It has been concluded therefore that zinc is a structural component of the enzyme.

4. Yeast Alcohol Dehydrogenase.

Vallee and Hoch (4) found yeast ADH to be a functional component of the apoenzyme molecule. It appears that four

atoms of zinc are present in one molecule of ADH apoenzyme. the zinc in the crystalline preparations is firmly bound; the zinc protein bond is maintained against competitive physio-chemical factors involved in fractionation, capable of dissociating it. Recrystallization or dialysis against water fail to remove zinc.

5. Glutamic Dehydrogenase.

Several crystalline preparations of beef liver glutamic dehydrogenase were examined (6) and zinc was found to be an integral part and functional component of the enzyme molecule. Based on molecular weight the number of gram atoms of zinc per mole of enzyme protein varied between four and five. Activity-zinc ratio increased with purification while the content of other metals decreased.

6. Lactic Dehydrogenase.

Zinc is thought to be a functional component of LDH of rabbit muscle (5). When metal binding agents were added to a preparation of the enzyme, zinc and the activity of the enzyme were inhibited. When zinc was added with the agent, however, the activity continued. As yet no zinc to protein ratio has been established for this enzyme.

7. Other Enzymes.

Enolase was found by Malmstrom (115) to be activated by zinc. One metal ion per molecule of enzyme caused activation, while more than one caused inhibition.

Various workers have investigated the possibility that uricase is a zinc-proteid. Holmberg (116) reported that

uricase contained 0.13 per cent zinc. Davidson (117) confirmed only 0.09 per cent zinc. Praetorius (118) doing studies with B.A.L.[1] found that the zinc in uricase combined with the B.A.L. instantaneously with the formation of a stable compound. He concluded that uricase was probably not a zinc proteid.

Presence of zinc as an active constituent of carnosinase (119), dehydropeptidase (120), and glycyl-L-leucine dipeptidase (121), as well as a pure yeast polypeptidase (122) has not been conclusively proven.

1. Dimercaprol (2, 3-dimercaptopropanol)

3. METHODS

A. Animals.

Male albino rats of the Wistar strain, weighing from 240-300 grams, were used in adrenalectomy and cortisone experiments. Adrenalectomy was performed by the dorsal route and animals were maintained on Miracle dog food pellets[1] and saline (1%). Rats of the sprague-Dawley strain, hypophysectomized by the transpharyngeal route, were obtained from the Hormone Assay Laboratories[2]. Normal rats for control purposes were obtained from the same source. The hypophysectomized rats were maintained on a diet or oranges, white bread and dog pellets, with water ad libitum.

Macroscopic examination was made on several hypophysectomized animals and there was no evidence of pituitary tissue. The adrenal glands showed the typical atrophy associated with hypophsectomy.

The animals were fasted for 24 hours before the experiment. In order to rule out numerous small sources of variation, control animals were examined with each group of test animals.

At the time of killing the animals were anesthetized with ether. A vertical incision was made up the abdomen to beyond the diaphragm. A sample of blood was withdrawn directly from the heart with a #18 needle into a 2 ml. hypodermic syringe, containing a small amount of heparin. The sample was then placed in a tared crucible.

The liver, adrenals, testis and the whole prostate gland were removed, blotted to remove adhering blood and

1. Ogilvie Flour Mills Co. Ltd.
2. Hormone Assay Lab. Inc., Chicago, Ill. U.S.A.

cleaned of adipose tissue. The prostate was separated into the dorsolateral and ventral portions.

The wet weight of the tissue samples was determined, the samples dried at 100°C for one hour and then dry-ashed in a muffle furnace for six hours at 600°C.

B. Injections.

Injections of cortisone were given in the experiments called chronic experiments for a period of fourteen days with the last dose being given 24 hours prior to killing. The chronic dose consisted of an intraperitoneal injection of 12.5 mg. of cortisone per day. This dose was the same as that used by Gorby, Leonard, Ambrus, and Harrison (123). In the acute experiments a single dose of 25 mg. of cortisone was given 24 hours before killing.

ACTH was given as both acute and chronic administrations to normal and hypophysectomized animals. Acute dosage consisted of an intraperitoneal injection of 4 mg. per 100 grams body weight, given four hours prior to killing as was used previously by Riedel, Logan and Rossiter (124).

Chronic dosage to both normal and hypophysectomized animals was 0.4 mg. per day by intraperitoneal injection with the last dose being given 24 hours before killing (124).

Each animal was injected with radioactive zinc in the form of a neutralized solution, containing 70 uc. A specially radiated sample was used which had a very high specific activity (1 mc per mg. Zn)[1]. This injection was made under ether anesthesia into the external jugular vein 24 hours before killing.

1. Received from Dr. C.A. Mawson, Chalk River, Ontario.

C. Zinc Estimation.

In 1926, Fairhall (125), described three methods for the estimation of zinc in biological material. These were gravimetric or titrimetric, turbidimetric, and colorimetric procedures. Newer methods, such as fluorescence (126), spectrography (127) and polarography (128), have been used with varying success.

The procedure which has been used most extensively with reporducible results has been the dithizone method (129-132). Zinc reacts with diphenylthiocarbazone in weakly alkaline medium to form the keto complex which is soluble in carbon tetrachloride or chloroform giving a solution with a bright red color. In weakly acid solution the reaction depends on the concentration of dithizone in the organic solvent. Since many other metals react with dithizone it is necessary to use agents to prevent the interference of these metals. Usually sodium thiosulfate is used to form a complex with interfering copper, mercury, silver, bismuth, gold, lead or cadmium. Potassium cyanide is used to prevent the reaction of nickel and cobalt.

Histochemical techniques for the determination of zinc have not met with much success. Glick (133) described a method by which zinc is precipitated by nitroprusside and the precipitate is stained a deep purple by treatment with sulfide. Other investigators have used dithizone as a stain with better results (134-136).

The method of Vallee and Gibson (131), was chosen for

the estimation of zinc in the tissues to be studied. Considerable care was necessary to ensure that zinc-free chemicals, water and glassware were used throughout this procedure. At pH 5.5 and in the presence of tartrate solution and complex-forming buffer, dithizone combined with zinc in stoichiometric proportions to form zinc dithizonate, but did not combine with other metals which were present. The completion of the reaction was accompanied by a change in color of the dithizone from green to bright red. The color change occurred slowly and appeared to pass through an intermediary purple stage. The complete procedure for the determination of zinc is given in Appendix A.

A Beckman Model B. spectrophotometer was used for the colorimetric determinations.

Dithizone in carbon tetrachloride was found to have an absorption maximum at 625 mμ, while zinc dithizonate showed an absorption maximum at 515 mμ, but was transparent at 625 mμ. This is shown in Fig. 1. These readings do not agree entirely with those of Vallee and Gibson (131) who used wavelengths of 620 and 520 mμ. Vikbladh (68) used wavelengths of 620 and 535 mμ.

With the filters employed by Vallee and Gibson (131) in an Evelyn colorimeter the ratio of the optical densities of dithizone at 620 mμ and 520 mμ had a numerical value of 4.65.

With the Beckman spectrophotometer and taking reading at 515 and 625 mμ, it was found that the ratio was 5.94. These differences are insignificant and only indicate the use of different instruments.

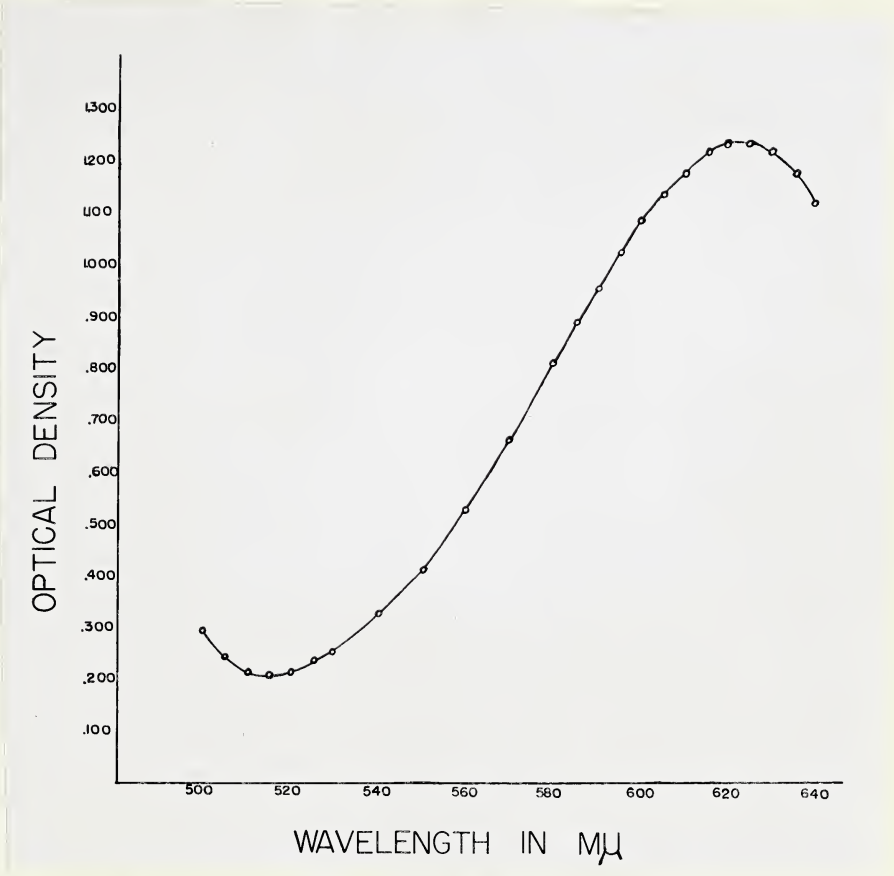


Figure I. Relationship between optical density and wavelengths for dithizone.

The zinc content of the samples was calculated from the following equation derived from Vallee and Gibson (131).

$$Zn = \left[\left(L515 - \frac{L625}{R}\right) \times D\right] - Blk \times K$$

L515 = optical density at 515 mμ.

L625 = optical density at 625 mμ.

R = ratio of optical density of dithizone in carbon tetrachloride at 625 mμ and 515 mμ.

D = dilution factor with relation to original volume.

Blk = blank determination.

K = calibration constant.

A stock solution of zinc chloride was prepared by dissolving 100 mgm. of zinc metal dust in concentrated HCl to make 100 ml. 10 ml. of this solution was transferred to a litre volumetric flask and made up to volume with zinc free water. Each ml. therefore contained 10 micrograms of zinc.

In order to establish that the zinc dithizonate followed the Lambert-Beer law, a series of standards containing 5 to 50 micrograms of zinc was prepared. These standards were extracted with dithizone and the total zinc dithizonate was made up initially to 50 ml. Further dilutions with carbon tetrachloride were made when necessary.

The results are presented in Figure 2 and a straight line relationship was observed over the range examined.

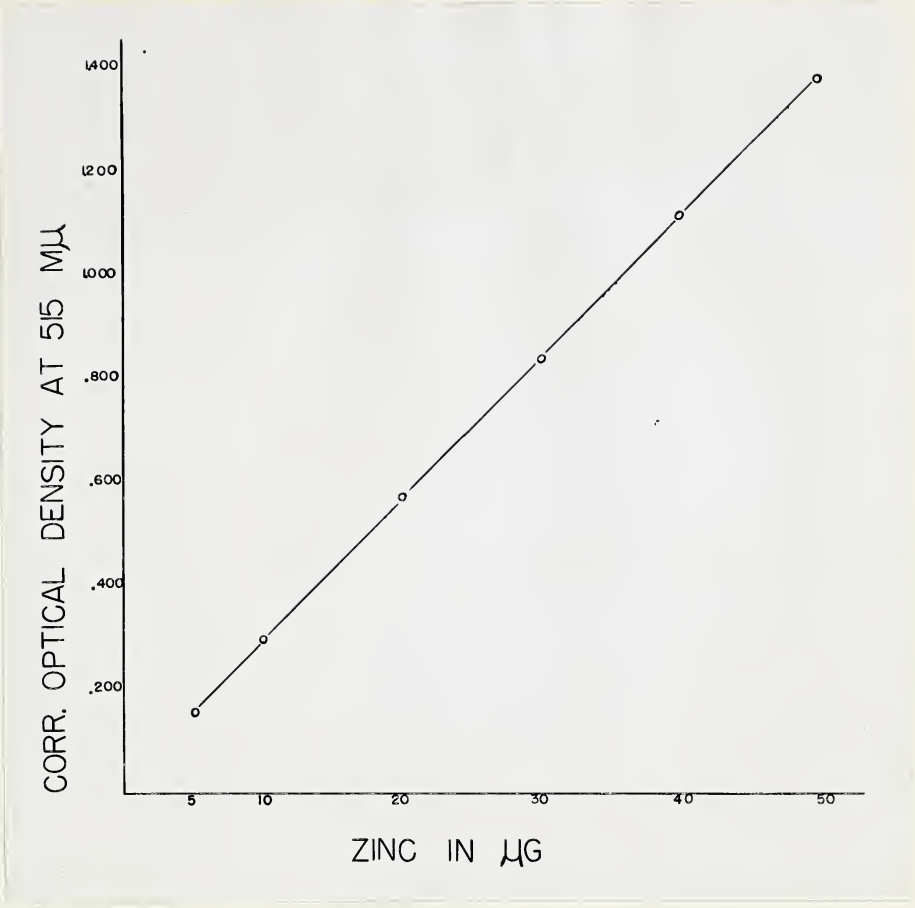
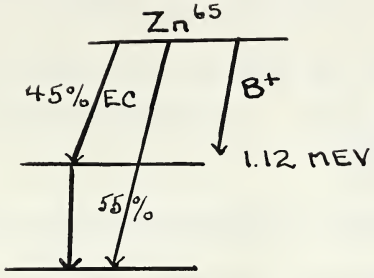


Figure 2. The relationship between corrected optical density at 515 mμ and concentration of Zn.

D. Determination of Radioactivity.

Zn^{65} is one of 13 known isotopes of zinc. The disintegration scheme may be shown as follows (137)



It has a half-life of 250 days.

The radioactivity due to the Zn^{65} in the samples was measured using a well scintillation counter[1]. The zinc dithizonate previously determined colorimetrically was extracted with 10 ml. of dilute H_2SO_4 and this acid solution used for counting.

Counts were recorded on a decade scaler[2].

The counting rate was corrected for background and decay. All counts were corrected to the time of the first experiment.

One dose as previously described (page 17) of the solution of Zn^{65} was taken for the preparation of a standard. This volume was diluted to 100 ml. and 100 λ transferred to a planchette for counting under a Geiger-Müller tube.

1. Atomic Instrument Company, Cambridge 39, Mass.
2. Tracerlab, Ottawa 2, Ontario.

E. Definition of Terms.

Specific Activity -

The specific activity of a sample was defined as the number of counts per minute per μg of zinc.

$$\text{Specific activity} - \frac{\text{net sample counting rate corrected for decay}}{\mu g \text{ Zn in the sample}}$$

The specific activity relative to the specific activity of the whole blood is defined by:

$$\text{Relative Specific Activity} = \frac{\text{Specific activity of sample}}{\text{Specific activity of whole blood}} \times 10^2$$

Corrected Specific Activity.

Since a constant amount of Zn^{65} was injected regardless of the size of the animals, it was necessary when blood samples from different animals were to be compared, to correct the specific activity for differences in dilution. The specific activity obtained for a particular rat was multiplied by the body weight at the time of killing to give a value (corrected specific activity) independent of animal size. These figures were reduced by a factor of 10^3 to make them more easily handled.

4. EXPERIMENTAL RESULTS

PART 1. Adrenalectomy and Cortisone Experiments.

The incorporation of Zn^{65} and the concentration of zinc were studied in rats under different hormonal conditions.

Rats adrenalectomized for a period of 14 days were compared with normal animals. Both normal and adrenalectomized rats were treated with cortisone in acute and chronic experiments as previously described (page 17).

Hypophysectomized animals were compared to normal animals either 5 or 19 days after hypophysectomy. Both normal control and test animals were treated with adrenocorticotrophic hormone in acute and chronic experiments (page 17).

Tissues studied were whole blood, liver, adrenals, testis, dorsolateral and ventral prostates.

The criteria for the conditions of the experiments and the administration of the hormones were previously established and discussed under the methods (pages 16 and 17).

A statistical evaluation and comparison of the results is presented for each tissue. The individual figures obtained for the tissues of each animal are presented in table form in Appendix B.

A. Whole Blood

Table 1 presents a statistical comparison of the effects of adrenalectomy and the administration of cortisone on the zinc concentration and Zn^{65} uptake of the blood.

Whole blood was used in these experiments. Adrenalectomy appeared to cause a marked decrease in the amount of zinc present but had no effect on the specific activity, corrected

TABLE I.

Whole Blood - Effect of Adrenalectomy and the Administration of Cortisone on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	BODY WT. IN GM.	ZN µG/GM WET WT.	SIG.	S/A CTS/µG ZN	CORRECTED S/A x 10^{-3}	SIG
Normal	24	240	10.8 ± 2.0	S	1134 ± 160	272 ± 38	S
Normal + cortisone (chronic)	10	245	11.5 ± 3.2		1034 ± 167	253 ± 41	
Normal + cortisone (acute)	7	260	5.5 ± 0.3	S	1129 ± 42	294 ± 11	
Adrenalectomized	9	240	6.6 ± 1.8	S	1082 ± 112	260 ± 27	S
Adrenalectomized + cortisone (chronic)	10	245	12.0 ± 3.5		1527 ± 107	373 ± 26	
Adrenalectomized + cortisone (acute)	8	240	4.6 ± 2.1		1481 ± 77	356 ± 19	

Individual figures are obtained from Appendix B.

The average figures are shown ± the S.D.M.

Significance is indicated as "S" only when the p value is less than .05 as determined by Student's "t" test.

Comparisons are made between the figures indicated by the ends of the bracket.

for animal weight variations. Cortisone, when administered as a single dose to normal animals caused a similar reduction in the amount of zinc but no effect was observed on the zinc^{65} uptake. Cortisone administered to adrenalectomized animals in chronic or daily dosage caused recovery to normal in the zinc level and a significant increase in the corrected specific activity. An acute dose of cortisone was effective in causing a similar increase in the corrected specific activity. The amount of zinc in the blood was found to agree closely with that published by Vallee and Altschule (35).

B. Liver.

In liver tissue there were no observable changes in normal animals (Table II). Adrenalectomy appeared to have no effect. With cortisone a decrease occurred in the zinc concentration with a single dose and an increase in the relative specific activity occurred in both the acute and chronic experiments.

C. Adrenals.

The administration of daily doses of cortisone resulted in a marked increase in the zinc concentration (Table III). This was not shown with a single dose. However, only the single dose caused a significant increase in the relative specific activity.

The amount of zinc in the adrenal glands is relatively high being about 5 times that of blood and 2-3 times that of the testis and anterior prostate.

D. Testis.

The amount of zinc in the testis is small. Only the administration of daily doses of cortisone caused any change

TABLE II

Liver - Effect of Adrenalectomy and the Administration of Cortisone on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A	REL. S/A	SIG.
Normal	17	38.4 ±8.2		2841 ± 105	255 ±27	
Normal + cortisone (chronic)	10	47.1 ±9.0		2400 ± 269	235 ±28.2	
Normal + cortisone (acute)	7	34.3 ±5.3		2801 ± 101	251.2±17.2	
Adrenalectomized	9	44.4 ±6.1		2497 ± 305	232 ±32	
Adrenalectomized + cortisone (chronic)	10	44.5 ±6.3	S	5000 ± 870	326 ±85	S
Adrenalectomized + cortisone (acute)	8	35.5 ±6.5		4669 ± 339	315 ±21	S

TABLE III

Adrenals - Effect of Adrenalectomy and the Administration of Cortisone on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A	REL. S/A	SIG.
Normal	14	59.2 ± 11.5	S	1500 ± 322	131 ± 36	S
Normal + cortisone (chronic)	10	106.7 ± 15.2		1144 ± 215	116 ± 31	
Normal + cortisone (acute)	7	66.8 ± 8.9		1955 ± 423	170 ± 32	

in concentration, a small but significant increase occurred (Table IV). A decrease in the relative specific activity was observed in normal animals given a single dose of cortisone.

E. Dorsolateral Prostate.

This tissue as previously observed (7) contained a large amount of zinc per unit weight (Table V). Adrenalectomy caused a reduction and both chronic and acute cortisone produced a marked increase in the amount present. The relative specific activity, however, was not altered by adrenalectomy although treatmeht with cortisone did result in an increase in adrenalectomized animals.

F. Ventral Prostate.

The ventral prostate differed considerably from the posterior prostate. The amount of zinc present was only about one-tenth that in the dorsolateral prostate (Table VI). The relative specific activity is only about one-half that in the dorsolateral prostate. Adrenalectomy had no effect on the zinc concentration or Zn^{65} uptake. The administration of cortisone had no effect on the concentration but was able to cause certain changes in the relative specific activity in both normal and adrenalectomized animals.

TABLE IV

Testis - Effect of Adrenalectomy and the Administration of Cortisone on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A	REL. S/A	SIG.
Normal	23	23.3 ± 3.6		933 ± 142	86 ± 20	
Normal + cortisone (chronic)	10	28.4 ± 4.9		876 ± 129	88 ± 23.5	S
Normal + cortisone (acute)	7	22.9 ± 1.4		728 ± 82.5	66 ± 5.6	
Adrenalectomized	9	22.1 ± 3.3	S	849 ± 103	69 ± 14	
Adrenalectomized + cortisone (chronic)	10	28.4 ± 4.9		1289 ± 241	87 ± 31	
Adrenalectomized + cortisone (acute)	8	23.5 ± 2.0		835 ± 31	57.4 ± 6.3	

TABLE V

Dorsolateral Prostate - Effect of Adrenalectomy and the Administration of Cortisone on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A	REL. S/A	SIG.
Normal	23	190 ± 41.3	S	2746 ± 361	249 ±52	S
Normal + cortisone (chronic)	10	211.1 ± 32		2637 ± 154	265 ±54	
Normal + cortisone (acute)	7	212.3 ± 20.3		2414 ± 178	215.6 ±22	
Adrenalectomized	9	139 ± 23	S	2216 ± 492	206 ±43	S
Adrenalectomized + cortisone (chronic)	10	195 ± 35	S	4287 ± 540	301 ±37.5	
Adrenalectomized + cortisone (acute)	8	226 ± 41		2733 ± 151	186.2 ±11	

TABLE VI

Ventral Prostate - Effect of Adrenalectomy and the Administration of Cortisone on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A	REL. S/A	SIG.
Normal	25	19.6 ± 4.2		1253 ± 206	112 ± 23	S
Normal + cortisone (chronic)	10	18.6 ± 4.5		1469 ± 302	144 ± 22.9	S
Normal + cortisone (acute)	7	17.1 ± 4.2		1268 ± 200	121.8 ± 5.0	
Adrenalectomized	9	17.0 ± 6.2		1069 ± 219	100.0±26	S
Adrenalectomized + cortisone (chronic)	10	17.8 ± 4.2		1891 ± 309	129.0±33	S
Adrenalectomized + cortisone (acute)	8	14.0 ± 2.0		1854 ± 200	125.0±13	S

Discussion of Adrenalectomy and Cortisone Experiments

The tissues of principle interest in this study were the sex glands of the rat. It was found that the dorsolateral prostate gland contained 10 times more zinc than the ventral prostate; 5 times more than the liver; 8 times more than testis; and approximately 20 times more than whole blood. These findings agree closely with those of Mawson and Fischer (7) for prostate, liver and testis, and those reported by Vallee and Altschule (35) for blood in the rat. The difference in zinc concentration between the dorsolateral and ventral prostate which was observed by Mawson and Fischer (7) was verified. This observation points up the fact that the dorsolateral and ventral prostates are two entirely different glands.

Zinc concentration decreased after adrenalectomy in both dorsolateral prostate and blood. Vikbladh (68) reported a decrease in zinc concentration in serum in Addison's disease and elevated levels when either cortisone or desoxycorticosterone were instituted as therapy. By adrenalectomizing, experimental Addison's disease has been produced with an accompanying fall in blood zinc concentration and a return to normal levels after therapy with cortisone during the period of adrenalectomy.

Although the concentrations of zinc were altered, the relative specific activity of the tissues and the corrected specific activity of the blood were not altered significantly by adrenalectomy. This would indicate that the metabolism

of Zn^{65} in an adrenalectomized animal (14 days after adrenalectomy) was in the same ratio as in a normal animal. This would also suggest that the mechanism responsible for the control and utilization of zinc in tissues was still functioning or that it had been replaced by some auxiliary mechanism.

When adrenalectomized animals were treated with cortisone acetate the relative specific activities of the tissues and the corrected specific activity of the blood were increased significantly in all tissues but testis. Cortisone therefore seemed to cause an increased uptake by all the tissues or, alternatively, a decrease in backflow from the tissues. The cortisone, therefore, acted either as a stimulant if the uptake was increased, or a depressant if the retention was greater.

Zinc concentration returned to normal in dorsolateral prostate and blood after therapy with cortisone.

Acute doses of cortisone increased the Zn^{65} concentration in blood, liver, and ventral prostate. Dorsolateral prostate did not exhibit an increase in Zn^{65} under these conditions. This was probably due to the large increase in zinc concentration which masked the increase in Zn^{65}.

The increased amount of radioactive zinc in the blood would probably indicate that the Zn^{65} was being retained in the blood or more backflow from tissues was occurring.

Zinc concentration and specific activities were not affected to any extent by cortisone injections in normal animals. Only in the adrenals was the relative specific

activity affected after acute treatment with cortisone. The retention of Zn^{65} by the adrenal was probably increased.

After chronic dosage there was a very marked increase in zinc concentration in the adrenals. Cortisone is known to inhibit the adrenal and the formation of corticoids is decreased. It would appear that the zinc in the gland is selectively concentrated. It is possible that the buildup of zinc indicates an association of zinc with corticoid formation. Further evidence for such an association is given in the experiment on hypophysectomized animals.

After chronic treatment with cortisone, the Zn^{65} concentration of the ventral prostate was increased in normal animals. The dorsolateral prostate did not exhibit this effect.

It would appear that in normal animals, in the presence of the adrenal glands, cortisone had neither a stimulating nor an inhibitory effect on the Zn^{65} metabolism.

Zinc concentration was affected in blood where a decrease was observed after acute treatment with cortisone. In the adrenals after chronic treatment with cortisone an increase was observed.

It may be concluded that the adrenals possess some control over the Zn^{65} levels in the tissues studied. In the absence of the adrenals, cortisone produced a reversal of the adrenalectomy effect in these tissues. Cortisone either stimulated all tissues to increase the amount of Zn^{65} uptake or inhibited the backflow from the tissues. The mechanism whereby such an effect occurs is not known.

RESULTS

Part II - Hypophysectomy and ACTH Experiments.

A statistical evaluation and comparison of the results is presented for each tissue. The individual figures obtained for the tissues of each animal are presented in table form in Appendix C.

A. Blood:

Table VII shows a statistical comparison of the effects of hypophysectomy and the administration of ACTH on the zinc concentration and Zn^{65} uptake of the blood.

Hypophysectomy appeared to have no effect on the amount of zinc present. An increase in the corrected specific activity was observed in the 5-day hypophysectomized animals. ACTH when administered as a single dose to normal animals did not cause any change in either the zinc concentration or the corrected specific activity. When a single dose was administered to hypophysectomized animals, however, a significant decrease in the corrected specific activity occurred. This was true of both the 5-day and the 19-day hypophysectomized animals.

ACTH administered daily over 14 days to normal and hypophysectomized animals caused a marked decrease in the zinc concentration. The corrected specific activity increased after chronic dosage in hypophysectomized animals.

B. Liver:

In the liver tissue there were no observable changes in the zinc concentration of normal animals (Table VIII). Hypophysectomy caused an increase in the zinc concentration

TABLE VII.

Whole Blood - Effect of Hypophysectomy and the Administration of ACTH on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	BODY WT. IN GM.	ZN µg/gm WET WT.	SIG.	S/A CTS/µg Zn	Corrected S/A x 10^{-3}	SIG.
Normal	24	240	10.8 ± 2.0	S	1134 ± 160	272 ± 38	S
Normal + ACTH (chronic)	7	240	3.47 ± .27		1158 ± 98	277 ± 27	
Normal + ACTH (acute)	7	237	10.2 ± 3.4		1143 ± 27	271 ± 13.5	
Hypox. (5 day)	12	169	10.1 ± 3.8		2009 ± 229	336 ± 29	S
Hypox. + ACTH (acute)	10	158	12.1 ± 1.8		1869 ± 162	308 ± 14.4	
Hypox. (19 day)	10	155	11.3 ± 1.5	S	1809 ± 118	278 ± 13	S
Hypox. + ACTH (chronic)	7	157	4.4 ± .83		1869 ± 283	293 ± 13	

Individual Figures obtained from Appendix C.

TABLE VIII

Liver - Effect of Hypophysectomy and the Administration of ACTH on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A CTS/µg Zn	REL. S/A	sig.
Normal	17	38.4 ± 8.2	S	2841 ± 105	255 ± 27	S
Normal + ACTH (chronic)	7	41.3 ± 3.6		2815 ± 139	245 ± 26	
Normal + ACTH (acute)	7	44.8 ± 5.1		3217 ± 223	282 ± 22	
Hypox. (5 day)	12	63.1 ± 15.9	S	5168 ± 789	260 ± 14.7	S
Hypox. + ACTH (acute)	10	62.3 ± 6		6241 ± 415	319 ± 33	
Hypox. (19 day)	10	62.1 ± 5.3	S	4886 ± 321	242 ± 18	S
Hypox. + ACTH (chronic)	7	24.4 ± 4.4		4809 ± 415	258 ± 12	

in the liver without a corresponding change in the relative specific activity. With ACTH an increase in specific activity was observed in 5-day hypophysectomized animals after acute dosage. After chronic dosage the relative specific activity of the liver was increased in both normal and hypophysectomized animals. A marked decrease in zinc concentration occurred in hypophysectomized animals after chronic dosage.

C. Adrenals:

The administration of ACTH to normal and hypophysectomized animals caused a decrease in the zinc concentration and an increase in the relative specific activity (Table IX). The zinc concentration of the adrenals in hypophysectomized animals decreased initially but after 19 days levels twice that of normal were obtained. Hypophysectomy for 19 days caused a decrease in the relative specific activity in adrenals. Treatment with ACTH in acute dosage resulted in a decrease in the relative specific activity.

D. Testis:

Hypophysectomy did not alter the zinc concentration but caused a decrease in the relative specific activity (Table X). The testis showed almost the same changes as the dorsolateral prostate (Table XI). An increase in the zinc concentration in normal animals and a decrease in hypophysectomized animals was observed after chronic treatment with ACTH. Hypophysectomized and normal animals treated with chronic ACTH both exhibited decreased relative specific activities. The ACTH cannot raise the level of the relative specific activity as it did in the ventral and dorsolateral prostate.

TABLE IX

Adrenals - Effect of Hypophysectomy and the Administration of ACTH on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A CTS/µg Zn	REL S/A	SIG.
Normal	14	59.2 ± 11.5	S	1500 ± 322	131 ± 36	S
Normal + ACTH (chronic)	7	11.7 ± 3		2751 ± 130	238 ± 16	
Normal + ACTH (acute)	7	67.7 ± 14.1	S	1492 ± 178	131.3 ± 16.7	S
Hypox. (5 day)	12	44.4 ± 10		2457 ± 193	122.6 ± 10.1	S
Hypox. + ACTH (acute)	10	52.5 ± 15.5	S	2136 ± 161	108.9 ± 18	
Hypox. (19 day)	10	128.5 ± 5.5		927 ± 84	51 ± 5.1	S
Hypox. + ACTH (chronic)	7	46.8 ± 10	S	1083 ± 134	58 ± 6.4	

TABLE X

Testis - Effect of Hypophysectomy and Administration of ACTH on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A CTS/µg Zn	REL S/A	SIG.
Normal	23	23.3 ± 3.6	S	933 ± 142	86 ± 20	S
Normal + ACTH (chronic)	7	27.3 ± 2.6		729 ± 53	63 ± 5.2	
Normal + ACTH (acute)	7	25.6 ± 2		848 ± 84	75 ± 7	S
Hypox. (5 day)	12	25.9 ± 3.5		1135 ± 91	58 ± 9.3	
Hypox. + ACTH (acute)	10	24.4 ± 4.1		1049 ± 103	54 ± 6	S
Hypox. (19 day)	10	22.0 ± 2.3	S	1192 ± 122	59 ± 6.9	S
Hypox. + ACTH (chronic)	7	13.3 ± 2.8		818 ± 75	44 ± 3	

E. Dorsolateral Prostate:

Hypophysectomy for 5 days caused a reduction in the amount of zinc as well as in the relative specific activity (Table XI). These changes were more marked after the 19-day hypophysectomy period. Chronic treatment of normal animals with ACTH caused a marked increase in zinc concentration and relative specific activity. The amount of zinc present was approximately 5 times that of normal.

Treatment of hypophysectomized animals with ACTH resulted in a further decrease of zinc concentration but the relative specific activity was increased to twice that of hypophysectomized (19-day) levels.

F. Ventral Prostate:

Ventral prostate is affected very little by hypophysectomy and ACTH administration. Chronic administration of ACTH caused a decrease of zinc concentration in both normal and hypophysectomized animals (Table XII). The relative specific activity was increased in the hypophysectomized animals after chronic ACTH administration. Hypophysectomy caused the relative specific activity to decrease markedly after 5 days with a further decrease after 19 days.

TABLE XI

Dorsolateral Prostate - Effect of Hypophysectomy and Administration of ACTH on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A CTS/µg Zn	REL S/A	SIG.
Normal	23	190 ± 41.3	S	2746 ± 361	249 ± 52	S
Normal + ACTH (chronic)	7	322 ± 32		3722 ± 183	303.6 ± 31	
Normal + ACTH (acute)	7	194 ± 17	S	2920 ± 100	255.5 ± 23	S
Hypox. (5 day)	12	160 ± 17.5	S	2163 ± 190	109 ± 10.3	S
Hypox. + ACTH (acute)	10	151.4 ± 15		2054 ± 209	104 ± 11	
Hypox. (19 day)	10	143.5 ± 16.5	S	467 ± 39	26 ± 3.3	S
Hypox. + ACTH (chronic)	7	60.6 ± 11.2		894 ± 72	48 ± 11	

TABLE XII

Ventral Prostate - Effect of Hypophysectomy and the Administration of ACTH on the Zn Concentration and Zn^{65} Incorporation

ANIMAL GROUP	NO. OF ANIMALS	Zn CONTENT µg/gm WET WT.	SIG.	S/A CTS/µg ZN	REL S/A	SIG.
Normal	25	19.6 ± 4.2	S	1253 ± 206	112 ± 23	S
Normal + ACTH (chronic)	7	8.6 ± 3		1202 ± 113	104.7 ± 15	
Normal + ACTH (acute)	7	17.3 ± 2.8		1171 ± 101	103 ± 5.7	S
Hypox. (5 day)	12	22.8 ± 3.4		1246 ± 127	63.8 ± 11.5	S
Hypox. + ACTH (acute)	10	25.8 ± 3.4		1128 ± 112	57.7 ± 7.7	
Hypox. (19 day)	10	22.1 ± 4.7	S	738 ± 72	41.1 ± 5.8	S
Hypox. + ACTH (chronic)	7	17.4 ± 3.4		1171 ± 94	63.3 ± 8.7	

Discussion of Hyphphysectomy and ACTH Experiments

Gunn and Gould (12) reported that the selective uptake of Zn^{65} by the dorsolateral prostate gland was decreased following hypophysectomy and could be maintained if gonadotrophin was administered. In the experiments reported here there was a decreased zinc concentration and an even more marked decrease in the Zn^{65} uptake following hypophysectomy. The effect was much greater than in any other tissue. This would suggest that the Zn^{65} uptake by the dorsolateral prostate is probably influenced by the pituitary gland. Chronic treatment with ACTH resulted in a two-fold increase in relative specific activity. This suggests that ACTH is responsible at least in part for zinc metabolism in this tissue.

Normal animals when treated with ACTH in chronic dosage exhibited a marked increase in the zinc concentration and Zn^{65} uptake. The ACTH caused a buildup of zinc in the dorsolateral prostate. The function of the zinc is not known but it seems possible that any increased storage of zinc takes place because other organs are not functioning to excrete the excess zinc or that the gland when stimulated can concentrate zinc beyond its normal capacity.

Hypophysectomy did not affect the zinc concentration of the blood. The corrected specific activity, however, was increased initially following hypophysectomy but returned to normal after the 19-day period of hypophysectomy. A period for readjustment of hormonal control was probably necessary. ACTH administered to normal and hypophysectomized animals in

chronic dosage caused a decrease in zinc concentration to about one-quarter the original value. A redistribution of zinc took place with the whole blood losing a part of its supply to other tissues or alternatively, the mobility of the zinc has increased leaving the amount of zinc present at a lower level. One dose of ACTH decreased the corrected specific activity in hypophysectomized animals while chronic dosage caused an increase in the corrected specific activity.

ACTH did not affect the zinc concentration of the liver of normal animals but did cause a decrease in the zinc concentration in hypophysectomized animals after chronic treatment. This change is the same as occurred in all other tissues studied. Hypophysectomy itself caused an increase in zinc concentration in the liver after both 5 and 19 days. The increased buildup in the liver following hypophysectomy along with the decreased concentration in dorsolateral prostate, and adrenal indicates that only the more active glands are affected by alteration in hormone balance resulting from removal of the pituitary. Upon treatment of hypophysectomized animals with ACTH in chronic dosage the zinc becomes more mobile and is no longer stored but rather is in a transient stage.

The uptake of Zn^{65} by normal and hypophysectomized animals was increased when ACTH was given in acute dosage or in chronic dosage to hypophysectomized animals.

The adrenal glands showed an initial decrease in zinc concentration following hypophysectomy with greatly

elevated levels 19 days after the operation. If the buildup of zinc in the adrenal glands of hypophysectomized animals indicates that corticosteroid formation is at a very low level, then it may be true that zinc takes part in adrenal corticosteroid formation. The low levels of zinc in the adrenals of normal animals after treatment with ACTH would further substantiate the above statement. The relative specific activity increased after chronic treatment with ACTH in both normal and hypophysectomized animals. If the gland is functioning at a high level the amount of Zn^{65} taken up by the gland may be increased or the backflow from the gland decreased.

Testis showed the same alteration in zinc concentration as the dorsolateral prostate. An increase in zinc concentration after chronic treatment of normal animals and a decrease after chronic treatment of hypophysectomized animals occurred. The ACTH caused the testis to take up less Zn^{65} or it may have increased the backflow from the tissue. Hypophysectomy also decreased the Zn^{65} uptake or increased the backflow from the tissue.

The relative specific activity of ventral prostate was decreased significantly following hypophysectomy after 5 days and decreased further after the 19 day period. The gland presumably became less able to concentrate the Zn^{65} which is present or has increased the amount leaving the gland. ACTH in chronic dosage caused the Zn^{65} levels to be increased. In the absence of the pituitary, therefore, the ACTH has the ability to increase Zn^{65} levels and may in some way influence the control mechanism of Zn^{65} uptake in the prostate glands.

5. SUMMARY

1. A study was made of the effects of adrenalectomy and hypophysectomy on zinc concentration and Zn^{65} incorporation.
2. Treatment of normal and experimental animals with adrenocorticotrophic hormone and cortisone was also undertaken.
3. The tissues studied were the whole blood, liver, adrenals, testis, dorsolateral and ventral prostates.
4. Adrenalectomy caused significant decreases in the zinc concentration of blood and dorsolateral prostate with a return to normal following chronic treatment with cortisone. This would indicate that the zinc concentration of the dorsolateral prostate is in some way partly controlled by the adrenal gland.
5. Cortisone caused increased levels of Zn^{65} in adrenalectomized animals in all tissues but testis following chronic administration of the hormone.
6. Hypophysectomy caused the zinc concentration to be decreased in dorsolateral prostate with a corresponding decrease in Zn^{65} incorporation. This may be due to absence of gonadotrophins but it may also be due in part to an absence of ACTH.
7. Chronic treatment of hypophysectomized animals with ACTH resulted in a lowering of zinc concentrations in all tissues but increased the Zn^{65} incorporation in

dorsolateral and ventral prostate.

8. Because of the dramatic changes observed in the zinc concentration of the adrenal with hypophysectomy and the administration of cortisone and ACTH it has been postulated that zinc is in some way associated with corticosteroid formation.
9. It is also postulated that the adrenal gland is associated with the control of zinc metabolism in the tissues studied. It has been previously shown that the gonadotrophins have a marked effect on zinc metabolism in the dorsolateral prostate. The work reported here, however, has shown that in the presence of the pituitary gland, changes in adrenal activity also result in certain changes in the dorsolateral zinc picture.

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A P P E N D I X A

DETAILED METHOD OF ZINC ANALYSIS

Animals and Tissue

1. Remove food, but not water (or saline) from animals 24 hours before killing.
2. Inject Zn^{65} at appropriate intervals before killing. Use the same syringe to inject one dose of Zn^{65} into a 100 ml volumetric flask for Zn^{65} standard.
3. Anesthetize with ether. Make a longitudinal incision along the abdomen and into the pleural cavity.
4. Remove approximately one ml of blood from the heart in a heparinized syringe.
5. Remove the adrenals, blot clear of adhering adipose and connective tissue.
6. Remove approximately 500 mgm. of liver from the central lobe, blot to remove adhering blood.
7. Remove the testis and prostate gland.
8. Separate the prostate into dorsolateral and ventral portions.
9. Weigh the tissues in tared crucible.

Analysis Method.

1. Dry the tissue for one hour at 100°C.
2. Ash the tissue for six hours at 600°C. in a muffle furnace.
3. Dissolve the ash by slow boiling in 2 N HCl.
4. Evaporate to a volume of 5 ml on a hot plate and transfer to a 125 ml Squibb separatory funnel by repeated washing with hot zinc free water.
5. Add 2 ml of tartrate solution.
6. Add 2 drops of methyl red solution.
7. Titrate to pH 5.5 with 0.1 N Nh_4OH (peach color).
8. Add 50 ml buffer solution and allow to stand until the color has completely faded.
9. Add about 10 ml of dithizone solution in carbon tetrachloride and shake vigorously for two minutes.
10. Draw off bottom layer (dithizone) into a 50 ml volumetric flask.
11. Repeat until the dithizone in the separatory funnel remains a clear green.
12. Add carbon tetrachloride to volume.
13. Carry out the same procedure with 5 ml of zinc free water in place of the sample to serve as a blank.
14. Read in a spectrophotometer at wavelengths of 515 and 625 mμ.
15. Return dithizone solution from the 50 ml volumetric flask to a Squibb separatory funnel.
16. Add 10 ml of dilute H_2SO_4.
17. Shake until dithizone is clear green again.
18. Draw off aqueous layer into 10 ml volumetric flask.
19. Count in scintillation counter.

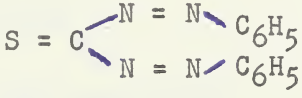
Reagents.

All reagents must be absolutely zinc free. The best grade of chemically pure reagents was obtained and even those may contain zinc in sufficient amounts to result in significant errors in the range of zinc content in which the worker was interested.

1. Zinc Free Water.

This was obtained by passing distilled water through a Bantam demineralizer (Barnstead Still and Sterilizer Co.)

2. Diphenylthiocarbazone (Dithizone)



100 mgm were dissolved in 1000 ml of carbon tetrachloride (previously redistilled). This stock solution of 10 mgm % was diluted to 1 mgm % for the extraction of small quantities of zinc.

3. Buffer Solution.

556 gm Sodium Thiosulfate
90 gm Sodium Acetate
10 gm Potassium Cyanide

The chemicals were dissolved in 1000 ml of zinc free water. The solution was then titrated to pH 5.5 with 15 N Acetic Acid. A sensitive pH meter was used to make the adjustment. The solution was then made up to 2000 ml with zinc free water in a volumetric flask. The buffer was then shaken with dithizone in carbon tetrachloride to remove any

contaminating zinc, the extraction being repeated in a 500 ml separatory funnel until the dithizone remains clear green.

4. Tartrate Solution.

A 20% solution of Sodium Potassium Tartrate C.P. (Rochelle Salts) was made up with zinc free water. This solution was shaken out with dithizone in the same manner as the buffer.

5. 0.1 N Nh_4OH

6. Concentrated NH_4OH

7. Dilute H_2SO_4

8. Methyl red indicator.

9. Zinc Solution.

100 milligrams of zinc metal dust were dissolved in concentrated hydrochloric acid to make 100 ml. 10 ml of this solution were transferred to a litre volumetric flask and made up to volume with zinc free water. (Each ml contains 10 µgm of zinc).

A P P E N D I X B

TABLE I

Blood - Normal Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

NORMAL				CORTISONE (Chronic)				CORTISONE (Acute)			
Animal Wt. (gm)	Zn µg/gm	S/A Cts/µg Zn	Corr. S/A	Animal Wt. (gm)	Zn µg/gm	S/A Cts/µgZn	Corr. S/A	Animal Wt (gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A
240	10.04	1225	294	245	16.33	1284	315	260	5.1	1090	283
245	14.9	1192	292	250	17.12	1120	280	260	5.2	1095	285
240	19.61	814	195	245	12.46	1310	321	260	4.79	1050	273
250	9.87	917	229	240	16.11	1248	299	260	7.16	1157	300
240	11.3	931	223	245	13.49	1135	278	260	7.71	1174	305
235	5.06	1115	262	240	9.49	852	205	260	4.8	1140	296
240	6.84	1250	300	245	8.15	932	228	260	6.01	1120	291
255	5.16	920	235	255	11.31	805	205				
240	7.17	1111	267	245	6.51	847	207				
230	7.56	1367	233	240	8.2	811	194				
240	6.07	1367	328								
250	7.53	1148	287								
240	6.7	1248	300								
245	5.0	1061	260								
240	8.16	1303	313								
225	14.1	1344	302								
240	7.7	1290	310								
	12.23										
	14.68										
	9.06										
	9.18										
	16.1										
	18.67										
	14.93										
Mean 240	10.8	1134	272	245	11.5	1034	253	260	5.5	1129	293.5
S.D	±2.0	±160	±38		±3.2	±167	±40.9		±0.3	±42	±10.9

TABLE II

Blood - Adrenalectomized Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

ADRENALECTOMIZED				CORTISONE (Chronic)				ADRENALECTOMIZED CORTISONE(Acute)			
Animal Wt.(gm)	Zn µg/gm	S/A cts/µg.	Corr. S/A	Animal Wt (gm)	Zn µg/gm	S/A cts/µg	Corr. S/A	Animal Wt.(gm)	Zn µg/gm	S/A cts/µg.	Corr. S/A
240	9.44	1070	257	245	12.06	1615	388	240	4.4	1481	355
240	5.67	1021	245	245	18.44	1220	293	240	3.6	1487	357
230	3.18	1239	284	230	11.44	1850	426	235	4.2	1513	355
260	6.41	906	236	260	12.64	1210	315	250	3.6	1302	326
240	6.39	1101	264	245	9.1	1269	305	240	9.6	1531	367
240	8.01	1096	263	255	9.49	1225	312	240	4.1	1482	355
250	5.24	911	278	220	3.8	2466	543	240	4.5	1375	329
230	9.41	1197	275	260	6.3	1231	320	235	2.7	1593	374
230	6.60	1196	275	245	15.2	1337	321				
				245	8.4	1846	443				
Mean 240	6.6	1082	259.7	245	12.0	1527	373.1	240	4.6	1481	355.5
S.D	±1.8	±112	±26.8		±3.5	±107	±26.2		±2.1	±77	±18.5

T A B L E III

Liver - Normal Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation

NORMAL			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
36.6	2770	226	50.0	2603	203	32.1	2881	274
26.2	3368	283	58.9	2438	217	24.9	2725	258
29.5	2714	333	56.9	2995	229	41.0	3026	274
24.6	2860	251	59.3	2615	209	34.5	2741	198
46.9	2334	312	43.1	2477	218	36.1	2577	228
47.0	3719	333	38.9	2118	249	41.0	2772	252
43.8	3092	247	38.6	2457	263	29.7	2872	266
35.5	2656	288	52.4	1995	247			
36.8	2880	259	32.8	1860	219			
49.2	2673	263	39.7	2435	300			
35.0	2571	188						
43.0	2470	215						
40.0	2744	214						
55.0	2705	254						
32.0	3071	226						
31.0	2537	188						
41.0	3127	242						
Mean 38.4	2841	255	47.1	2400	235	34.3	2801	251.2
S.D. ±8.4	±105	±27	±9.0	±269	±28.2	±5.3	±101	±17.2

T A B L E IV

Liver - Adrenalectomized Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

ADRENALECTOMIZED			CORTISONE (Chronic)			Cortisone (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
33.8	2650	247	51.1	5120	317	44.8	4984	336
43.0	2684	263	53.3	4590	244	40.6	4287	288
35.4	2295	185	43.6	5567	301	38.3	4095	337
46.4	2059	227	38.9	5813	480	30.5	5096	330
50.2	2733	247	43.1	5412	426	31.6	4179	267
48.5	2042	186	51.2	5270	430	32.1	5340	343
49.6	2475	272	37.0	6938	281	41.2	5075	287
48.9	3024	253	33.0	3152	256	23.8	4257	335
45.4	2513	210	48.1	4159	311			
			47.0	3980	216			
Mean 44.4	2497	232	44.5	5000	326	35.5	4669	315
S.D. ±6.1	±305	±32	±6.3	±870	±84.5	±6.5	±339	±21

T A B L E V

Adrenals - Normal Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

NORMAL			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
33.8	2009	164	115.0	1142	89	31.7	1333	122
58.8	2102	169	97.3	1244	111	58.9	1797	164
69.9	1688	207	117.3	606	46	74.7	1617	154
34.3			75.3	1619	129	63.2	1516	131
79.9	1461	131	128.7	1069	94	102.3	2810	239
55.9			112.2	1277	150	70.3	2284	200
65.9	1364	148	114.0	629	67	66.7	2325	208
60.3	987	89	89.7	1150	143			
66.6	1211	119	100.1	1482	175			
48.1	1159	84	114.6	1231	152			
45.4	1435	125						
35.7	1533	120						
67.5	1312	100						
83.6	1821	136						
83.3	1425	110						
Mean 59.2	1500	131	106.7	1144	116	66.8	1955	170
S.D. ± 11.5	± 322	± 36	± 15.2	± 215	± 31	± 8.9	± 423	± 32

T A B L E VI

Testis - Normal Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

NORMAL			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
21.5	1039	87	20.1	1033	80	23.8	775	74
19.7	1103	136	22.6	964	86	23.5	711	68
17.0	852	105	36.8	653	50	22.4	646	59
17.0	962	103	33.1	674	54	21.3	717	56
23.7	837	75	25.3	815	72	19.5	879	77
21.2	923	74	28.4	897	105	26.3	728	66
20.8	906	98	29.7	1132	121	22.8	646	60
22.6	923	83	34.7	717	89			
20.9	791	78	22.4	952	112			
18.2	914	67	26.5	923	114			
21.4	1327	116						
21.3	705	61						
29.5	859	81						
25.6	815	77						
27.6	1040	63						
26.8	944	73						
23.8								
26.4								
28.7								
29.2								
25.1								
22.2								
24.0								
Mean 23.3	933	86	28.4	876	88	22.9	728	66
S.D. ± 3.6	±142	±20	±4.9	±129	±23.5	±1.4	±82.5	±5.6

T A B L E VII

Testis - Adrenalectomized Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

ADRENALECTOMIZED			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
25.6	745	70	20.1	981	71	23.6	804	55
20.3	1052	103	35.7	717	69	24.5	1134	76
18.4	923	74	23.8	1070	68	23.3	836	56
20.0	751	83	24.6	627	61	22.0	808	62
26.7	955	87	38.0	489	45	26.7	745	49
22.5	686	63	34.0	848	81	24.1	757	52
26.0	818	90	27.1	2094	99	19.8	930	67
19.7	1055	88	27.5	1641	155	23.1	678	42
18.3	657	55	24.9	1147	100			
			26.9	1944	121			
Mean 22.1	849	69	28.4	1289	87	23.5	835	57.4
S.D. ±3.3	±103	±14	±4.9	±241	±31	±2.0	±31	±6.3

T A B L E VIII

Dorsolateral Prostate - Normal Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

NORMAL			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
195.9	2581	211	215.8	2725	212	193.3	2482	236.6
113.5	3401	199	243.7	2641	236	229.8	2277	211.4
253	3376	415	225.8	2800	214	212.1	2193	197.4
132	2625	286	230.4	2476	198	230.7	2199	165.2
203.8	3244	469	197.5	2562	226	236.0	2485	219.8
185.5	2437	219	225.1	2662	312	176.0	2632	239.4
221.1	3407	273	168.2	2332	250	207.5	2631	243.6
222.7	3093	387	258.2	2717	339			
239.4	2654	192	150.4	2476	292			
185.7	2889	285	196.1	2991	369			
167.4	2504	183						
192.1	3044	265						
211.6	2461	192						
247.6	2763	260						
242.4	2601	200						
205.9	2229	166						
184.2	2249	174						
190.4								
152								
123.4								
191.1								
193.6								
154.1								
Mean 190	2746	249	211.1	2637	265	212.3	2414	215.6
S.D. ±41.3	±361	±52	±32	±154	±34	±20.3	±178	±22

TABLE IX

Dorsolateral Prostate - Adrenalectomized Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

ADRENALECTOMIZED			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
133.6	2800	263	185.7	4367	316	193.2	2743	185
140.8	2622	257	249.4	3830	367	213.2	2535	169
114.6	1810	146	249.7	4569	288	208.2	2425	160
183.4	1382	153	218.8	3659	353	207.4	2764	211
142.8	1646	151	153.1	3510	324	277.1	2941	192
103.3	2268	207	151.8	3271	312	164.6	2576	174
155.2	2021	222	187.9	4715	224	289.8	2897	210
138.0	2654	222	210.6	3429	297	255.5	2988	188
127.3	2738	229	157.4	4458	283			
			181.9	2899	252			
Mean 139	2216	206	195	4289	301	226	2733	186.2
S.D. ± 23	± 492	± 43	± 35	± 540	± 37.5	± 41	± 151	± 11

T A B L E X

Ventral Prostate - Normal Animals

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation

NORMAL			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
16.1	791	65	23.4	1249	114	15.9	1270	118
12.1	1371	115	22.7	1585	165	11.9	1115	103
17.9	1132	139	17.7	1544	138	15.7	1236	112
28.5	1078	118	12.3	1914	179	19.7	1620	169
23.3	963	103	12.0	1121	115	19.7	1495	134
19.4	1497	134	18.7	1266	173	22.1	921	86
20.3	1213	97	24.5	952	119	14.7	1296	129
8.8	1276	139	16.1	913	133			
16.8	1106	100	24.1	1070	148			
19.8	1215	120	15.4	1089	157			
20.3	1459	107						
22.5	822	72						
20.0	1486	116						
23.0	1638	154						
17.9	1775	136						
21.1	1329	99						
20.5	1158	90						
21.1								
18.0								
15.2								
23.9								
16.7								
17.1								
25.5								
22.5								
Mean 19.6	1253	112	18.6	1469	144	17.1	1268	121.8
S.D. ± 4.2	± 206	± 23	± 4.5	± 302	± 22.9	± 4.2	± 200	± 5.0

T A B L E XI

Ventral Prostate - Adrenalectomized Animal

Effect of Cortisone on Zn Concentration and Zn^{65} Incorporation.

ADRENOLECTOMIZED			CORTISONE (Chronic)			CORTISONE (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
16.3	774	72	15.9	2091	129	15.9	2120	144
12.1	1026	100	10.9	2162	177	11.9	2026	136
27.5	762	61	16.5	1874	101	11.7	1689	111
23.7	923	102	12.8	2423	200	12.1	1626	125
17.3	1070	97	21.1	1680	132	12.2	2067	135
12.4	1130	103	21.3	1517	124	15.9	1594	104
10.8	1459	160	23.5	2413	97	15.2	1853	123
20.0	1258	105	15.3	1698	137			
14.3	1225	102	23.2	1819	98			
			16.5	1236	99			
Mean 17.0	1069	100	17.8	1891	129	14.0	1854	125
S.D. ± 6.2	± 219	± 26	±4.2	±309	±33	±2.0	±200	±13

A P P E N D I X C

TABLE I

Blood - Normal Animals

Effect of ACTH on Zn Concentration and Zn^{65} Incorporation.

NORMAL				ACTH (Chronic)				ACTH (Acute)			
Animal Wt.(gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A	Animal Wt.(gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A	Animal Wt.(gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A
				240	1.82	1223	293	240	5.6	1148	275
				250	2.8	1013	253	240	14.5	1120	269
				240	2.2	1279	307	240	14.5	1099	264
				250	4.6	1091	273	220	6.3	1319	290
				240	4.0	1045	251	240	10.2	1033	248
				230	5.0	1245	286	240	11.1	1156	277
				230	3.9	1210	278	240	9.4	1128	271
Mean 240	10.8	1134	272	240	3.47	1158	277	237	10.2	1143	271
S.D.	± 2.0	± 160	± 38		± .27	± 98	± 22		± 3.4	± 27	± 13.5

Values for normal animals obtained from Appendix B Table I.

TABLE II

Blood - Hypophysectomized Animals (5 day)

Effect of Acute ACTH on Zn Concentration and Zn^{65} Incorporation.

HYPOPHYSECTOMIZED				ACTH (Acute)			
Animal Wt.(gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A	Animal Wt. (gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A
160	6.2	2340	374	160	12.6	1926	308
160	8.9	2387	382	160	14.0	1964	314
180	17.2	1679	302	170	13.5	1772	301
170	11.6	1939	329	170	12.8	1690	287
180	9.9	1884	339	160	9.4	1832	293
180	4.6	1749	315	130	10.7	2598	337
160	10.8	2361	378	160	12.2	2034	325
180	17.0	1627	293	170	13.6	1734	295
160	10.8	2202	352	130	11.5	2402	312
170	11.3	1822	310	160	9.2	1914	306
170	6.7	1904	324				
160	6.4	2119	339				
Mean 169	10.12	2009	336	158	12.1	1986	307.8
S.D.	± 3.8	± 229	± 29		± 1.8	± 283	± 14.4

T A B L E III

Blood - Hypophysectomized Animals (19 day)

Effect of Chronic ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTIOMIZED				ACTH (Chronic)			
	Animal Wt. (gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A	Animal wt.(gm)	Zn µg/gm	S/A cts/µg Zn	Corr. S/A
	135	12.2	1916	259	160	4.5	1725	276
	155	10.0	1752	271	160	4.1	1795	287
	135	9.1	1946	263	160	3.2	1766	283
	135	13.6	1980	267	140	3.8	2242	314
	150	10.5	1920	288	160	4.1	1880	300
	175	10.7	1731	303	160	6.1	1814	290
	160	9.8	1792	287	160	4.9	1862	298
	160	11.6	1757	281				
	170	9.7	1682	286				
	170	13.9	1610	274				
Mean	155	11.3	1808.6	277.9	157	4.39	1869	293
S.D.		± 1.5	± 118	± 13		± .83	± 162	± 13.3

T A B L E IV

Liver - Normal Animals

Effect of ACTH on Zn Concentration and Zn^{65} Incorporation.

	NORMAL			ACTH (Chronic)			ACTH (Acute)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
				42.0	2695	220	48.4	3116	271
				39.1	3050	301	48.1	3611	322
				40.8	2936	229	29.3	2924	266
				41.2	2616	240	38.0	3495	265
				37.6	2718	260	50.3	3184	308
				49.9	2856	229	43.2	3129	271
				38.4	2834	234	47.6	3058	271
Mean	38.4	2841	255	41.3	2815	244.7	44.8	3217	282
S.D.	± 8.4	± 105	± 27	± 3.6	± 139	± 26	± 5.1	± 223	± 22

Values for normal animals obtained from Appendix B Table III.

T A B L E V

Liver - Hypophysectomized Animals (5 day)

Effect of Acute ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Acute)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	66.7	6155	263	55.2	6896	358
	52.3	6408	268	59.4	6761	344
	47.3	5462	325	55.8	6351	359
	63.1	5935	306	59.2	5551	328
	81.3	3852	205	58.0	6458	353
	40.6	4506	258	70.2	6361	245
	53.9	5023	213	63.5	5944	292
	46.6	3976	244	59.9	5616	324
	89.2	5132	233	74.2	6475	270
	63.7	5703	313	67.4	5987	313
	89.6	4519	237			
	63.2	5339	252			
Mean	63.1	5168	259.8	62.3	6241	319
S.D.	± 15.9	± 789	± 14.4	± 6	± 415	± 33

T A B L E VI

Liver - Hypophysectomized Animals (19 day).

Effect of Chronic ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Chronic)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A Cts/µg Zn	Rel. S/A.
	59.5	5109	238	29.3	4469	259
	62.7	4501	229	24.5	4950	276
	65.3	4925	226	16.6	4667	264
	52.7	4493	202	24.3	5395	240
	63.3	5494	255	20.2	4579	243
	65.9	5162	266	26.6	4752	262
	71.0	4795	238	29.3	4853	260
	66.3	5139	261			
	60.4	4738	251			
	54.1	4503	249			
Mean	62.1	4886	241.5	24.4	4809	257.7
S.D.	± 5.3	± 321	± 18	± 4.4	± 287	± 11.8

T A B L E VII

Adrenals - Normal Animals

Effect of ACTH on Zn Concentration and Zn^{65} Incorporation.

	NORMAL			ACTH (Chronic)			ACTH (Acute)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
				14.6	2841	232	52.3	1758	153
				9.3	2527	249	97.0	1690	151
				11.4	2936	230	70.8	1252	114
				8.0	2616	240	59.6	1374	105
				16.0	2718	260	74.3	1344	130
				13.5	2856	229	62.4	1452	126
				9.4	2762	228	57.5	1572	140
Mean	59.2	1500	131	11.7	2751	238	67.7	1492	131.3
S.D.	± 11.5	± 322	± 36	± 3	± 129.5	± 16	± 14.1	± 178	± 16.7

Values for normal animals obtained from Table V Appendix B.

T A B L E VIII

Adrenals - Hypophysectomized Animals (5 day)

Effect of Acute ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Acute)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	47.9	2955	126	30.5	2430	126
	33.7	3058	128	65.3	2254	115
	38.0	2253	134	56.4	1986	112
	40.0	2461	127	34.6	2356	139
	68.0	2109	112	73.5	1922	105
	32.3	2154	123	61.6	2170	84
	34.5	2891	123	73.9	2026	100
	35.4	1685	103	52.0	2194	117
	43.2	2509	114	42.1	1794	75
	50.2	2435	134	34.2	2228	116
	48.9	2536	133			
	61.2	2436	115			
Mean	44.4	2457	122.6	52.5	2136	109
S.D.	± 10	± 193	± 10.1	± 15.5	± 161	± 18

T A B L E IX

Adrenals - Hypophysectomized Animals (19 day)

Effect of Chronic ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Chronic)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/Gm	S/A cts/µg Zn	Rel. S/A.
	133.0	976	51	33.5	1239	72
	137.2	814	44	45.7	911	51
	101.2	1041	53	64.7	949	54
	143.0	991	50	51.2	1286	57
	133.9	817	43	47.9	1080	57
	121.0	805	47	38.8	983	54
	124.7	1005	56	45.7	1133	61
	135.4	917	52			
	145.5	1004	60			
	110.1	903	56			
Mean	128.5	927.3	51.2	46.8	1083	58
S.D.	± 5.5	±84	± 5.1	±10	±134	±6.4

T A B L E X

Testis - Normal Animals

Effect of ACTH on Zn Concentration and Zn^{65} Incorporation.

NORMAL			ACTH (Chronic)			ACTH (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
			28.4	838	68	26.4	886	77
			29.8	733	72	26.8	1017	91
			26.8	768	60	27.4	823	75
			21.0	680	62	22.3	736	56
			28.2	670	64	24.5	782	76
			29.5	690	55	26.4	824	71
			27.3	723	60	26.3	869	77
Mean 23.3	933	86	27.3	729	63	25.6	848	74.7
S.D. ± 3.6	± 142	± 20	± 2.6	± 53	± 5.2	± 2	± 84	± 7

Values for normal animals obtained from Table VI Appendix B.

T A B L E XI

Testis - Hypophysectomized Animals (5 day)

Effect of Acute ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Acute)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	26.2	1059	45	21.8	1015	53
	25.3	1216	51	27.7	1014	52
	30.3	1229	73	17.1	1194	66
	26.3	1263	65	18.4	965	55
	18.4	1063	56	27.5	826	45
	24.3	1031	59	23.6	1061	45
	28.9	1090	46	27.4	1082	54
	14.4	1236	76	23.7	1050	59
	33.1	1006	46	26.8	1187	53
	25.2	1204	66	28.6	1099	58
	27.6	1187	62			
	31.1	1036	49			
Mean	25.9	1135	57.8	24.4	1049	54
S.D.	± 3.5	± 91	± 9.3	± 4.1	± 103	± 6.0

T A B L E XII

Testis - Hypophysectomized Animals (19 day)

Effect of Chronic ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Chronic)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	24.0	1012	48	13.1	771	44.6
	25.3	1029	52	10.2	878	48.9
	18.0	1181	55	16.8	831	47.0
	19.1	1381	61	12.6	957	72.6
	20.5	1139	53	13.8	700	37.2
	23.4	1093	56	14.6	798	43.9
	23.5	1335	67	11.9	789	42.3
	19.4	1242	63			
	22.3	1231	65			
	24.0	1281	70			
Mean	22.0	1192	59	13.3	817.7	43.9
S.D.	± 2.3	± 122	± 6.9	± 2.8	± 75	± 3

TABLE XIII

Dorsolateral Prostate - Normal Animals

Effect of ACTH on Zn Concentration and Zn ^{65} Incorporation.

NORMAL			ACTH (Chronic)			ACTH (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
			308.9	3516	287	173.0	2408	210
			256.3	3759	371	197.9	3185	284
			277.6	3931	306	175.4	2719	247
			325.0	3609	331	221.0	3407	258
			349.7	3451	330	196.1	2887	279
			319.0	3963	318	182.5	2978	258
			287.6	3824	316	211.7	2858	253
Mean 190	2746	249	303.6	3722	322	194.0	2920	255.5
S.D. ± 41.3	± 361	± 52	± 31	± 183	± 32	± 17	± 100	± 23

Values for normal animals obtained from Table VIII Appendix B.

T A B L E XIV

Dorsolateral Prostate - Hypophysectomized Animals (5day)

Effect of Acute ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Acute)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	183.8	2265	97	123.6	2154	112
	147.6	2442	102	146.5	2166	110
	163.6	2084	124	142.9	1972	111
	175.0	1963	101	141.8	1874	111
	154.4	2036	108	149.2	1808	99
	167.0	2199	126	159.0	2162	83
	119.6	2230	94	141.6	2282	112
	152.8	1991	122	161.3	1660	96
	179.3	2414	110	183.4	2360	98
	164.9	1926	106	164.9	2102	110
	138.7	1941	102			
	172.0	2462	116			
Mean	160	2163	109	151.4	2054	104
S.D.	± 17.5	± 190	± 10.3	± 15	± 209	± 11

T A B L E XV

Dorsolateral Prostate - Hypophysectomized Animals (19 day)

Effect of Chronic ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Chronic)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	140.1	512	27	45.0	804	47
	103.7	417	24	54.8	969	54
	162.5	452	23	58.8	902	51
	155.5	457	23	86.4	989	44
	139.6	444	23	56.9	812	43
	159.8	435	25	63.6	872	48
	128.8	422	24	57.6	913	49
	154.9	521	30			
	147.1	477	28			
	143.8	533	33			
Mean	143.5	467	26	60.6	894	48
S.D.	± 16.5	± 39	± 3.3	± 11.2	± 71.5	± 3.5

T A B L E XVI

Ventral Prostate - Normal Animals

Effect of ACTH on Zn Concentration and Zn^{65} Incorporation.

NORMAL			ACTH (Chronic)			ACTH (Acute)		
Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
			8.5	1127	92	18.7	1063	93
			14.4	1378#	136	21.8#	1158#	103
			4.8#	1361	106	17.1	1029	94
			6.0	1215	111	17.1	1312	100
			6.0	1115	107	12.4	1226	118#
			12.1	1117	90	16.3	1243	108#
			8.4	1102	91	18.2	1171	104
Mean 19.6	1253	112	8.6	1202	104.7	17.3	1171	103
S.D. ± 4.2	± 206	± 23	± 3	± 113	± 15	± 2.8	± 101	± 5.7

Values for normal animals obtained from Table X Appendix B.

T A B L E XVII

Ventral Prostate - Hypophysectomized Animals (5 day)

Effect of Acute ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Acute)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	28.4	1016	43	23.5	1231	64
	22.9	1120	47	26.5	938	48
	22.0	1572	93	21.5	1035	58
	21.8	1240	64	28.7	1087	64
	22.2	1142	61	23.5	1042	57
	28.8	1279	73	21.1	1184	46
	23.2	1314	56	29.1	1237	61
	21.1	1202	74	24.0	1297	75
	24.7	1313	60	32.8	1204	50
	17.8	1233	68	24.6	1026	54
	20.4	1327	70			
	20.7	1188	56			
Mean	22.8	1246	63.8	25.8	1128	57.7
S.D.	± 3.4	± 127	± 11.5	± 3.4	± 112	± 7.7

T A B L E XVIII

Ventral Prostate - Hypophysectomized Animals (19 day)

Effect of Chronic ACTH on Zn Concentration and Zn^{65} Incorporation.

	HYPOPHYSECTOMIZED			ACTH (Chronic)		
	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.	Zn µg/gm	S/A cts/µg Zn	Rel. S/A.
	29.4	708	37	21.3	1244	72
	22.8	714	41	13.2	1285	72
	22.5	713	37	15.7	1261	71
	22.8	610	31	12.7	1036	46
	18.7	739	38	19.0	1188	63
	14.9	795	46	21.2	1061	59
	16.2	759	42	19.2	1123	60
	28.6	841	48			
	26.3	652	39			
	18.7	845	52			
Mean	22.1	737.6	41.1	17.4	1171	63.3
S.D.	± 4.7	± 72	± 5.8	± 3.3	± 94	± 8.7

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